

I claim:

1. A method for identifying a sequence of nucleotides in a polynucleotide, the method comprising the steps of:
 - (a) extending an initializing oligonucleotide along the polynucleotide by ligating an oligonucleotide probe thereto to form an extended duplex;
 - (b) identifying one or more nucleotides of the polynucleotide; and
 - (c) repeating steps (a) and (b) until the sequence of nucleotides is determined.
2. The method of claim 1 wherein said oligonucleotide probe has a chain-terminating moiety at a terminus distal to said initializing oligonucleotide.
3. The method of claim 2 wherein said step of identifying includes removing said chain-terminating moiety and extending said oligonucleotide probe with a nucleic acid polymerase in the presence of one or more labeled chain-terminating nucleoside triphosphates.
4. The method of claim 3 further including a step of regenerating an extendable terminus on said extended duplex.
5. The method of claim 4 wherein said oligonucleotide probe includes a subsequence of four ribonucleotides and wherein said step of regenerating includes cleaving said oligonucleotide probe with RNase H.
6. The method of claim 5 wherein said chain-terminating moiety is a 3' phosphate.
7. The method of claim 2 further including a step of capping an extended duplex or said initializing oligonucleotide whenever the extended duplex or said initializing oligonucleotide fails to ligate to said oligonucleotide probe.

8. The method of claim 2 further including a step of regenerating an extendable terminus on said extended duplex. ✓

9. The method of claim 8 wherein said step of regenerating includes
5 cleaving a chemically scissile internucleosidic linkage in said extended duplex.

10. The method of claim 9 wherein said chemically scissile internucleosidic linkage is a phosphoramidate.

11. The method of claim 8 wherein said step of regenerating includes enzymatically cleaving an internucleosidic linkage in said extended duplex.

12. The method of claim 11 wherein said oligonucleotide probe includes
15 a subsequence of four ribonucleotides and wherein said step of regenerating includes cleaving said oligonucleotide probe with RNase H.

13. A method for determining the nucleotide sequence of a polynucleotide, the method comprising the steps of:

- 20 (a) providing a template comprising the polynucleotide;
(b) providing an initializing oligonucleotide which forms a duplex with the template adjacent to the polynucleotide;
(c) annealing an oligonucleotide probe to the template adjacent to the initializing oligonucleotide;
25 (d) ligating the oligonucleotide probe to the initializing oligonucleotide to form an extended duplex;
(e) identifying one or more nucleotides of the polynucleotide by a label on the ligated oligonucleotide probe; and
(f) repeating steps (c) through (e) until the nucleotide sequence of
30 the polynucleotide is determined.

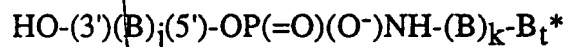
14. The method of claim 13 wherein said oligonucleotide probe has a chain-terminating moiety at a terminus distal to said initializing oligonucleotide and wherein said method further includes a step of
35 regenerating an extendable terminus on said oligonucleotide probe.

15. The method of claim 14 further including a step of capping said extended duplex or said initializing oligonucleotide that fails to ligate to said oligonucleotide probe.

5 16. The method of claim 14 wherein said step of identifying consists of identifying a single nucleotide of said polynucleotide.

17. The method of claim 16 wherein said step of identifying includes removing said chain-terminating moiety and extending said oligonucleotide probe with a nucleic acid polymerase in the presence of one or more labeled chain-terminating nucleoside triphosphates.

18. An oligonucleotide probe of the formula:



wherein:

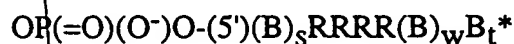
B is a nucleotide or an analog thereof;

j is in the range of from 1 to 12;

20 k is in the range of from 0 to 12, such that the sum of j and k is less than or equal to 12; *non-extendable*

B_t^{*} is a labeled chain-terminating moiety.

25 19. An oligonucleotide probe selected from the group consisting of:



35 and



wherein:

B is a deoxyribonucleotide or an analog thereof;

133

R is a ribonucleotide;
s is in the range of from 1 to 8;
w is in the range of from 0 to 8, such that the sum of j and k is less
than or equal to 8;
B_t* is a labeled chain-terminating moiety.

5

260730. 9442230